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Mechanoelectrical transducer has discrete conductances in the chick vestibular hair cell

(mechanoreceptor/transduction current/patch clamp)

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ABSTRACT **Properties of mechanoelectrical transduction were studied at the single-cell level by applying a whole-cell recording variation of the patch-clamp technique to dissociated vestibular hair cells of chicks. The hair bundle was directly stimulated by a glass rod, and transduction currents were recorded from the cell body. After a triangular movement of the stimulating probe, the transduction current was generated stepwise between discrete levels of amplitude. The minimum step amplitude was −1.8 pA at −27 mV in Na-containing normal saline.**

Hair cells in the inner ear and in the lateral line sensory organs are receptors for mechanical stimuli and generate electrical signals for the further processing in the central nervous system. Unique features of transduction mechanism have been studied by observation of microphonic potentials and by some direct observations of intracellularly recorded receptor potentials (1, 2). Recently, the whole-cell recording variation of the patch-clamp technique has been applied to dissociated hair cells, and properties of ionic currents have been studied (3, 4). In this paper, the whole-cell recording technique was extended to an analysis of the mechanoelectrical transduction current generated by microstimulations of the hair bundle of a dissociated hair cell from the chick vestibular organ.

MATERIALS AND METHODS

Hair cells were dissociated by an essentially similar procedure as the technique described for photoreceptor cells (5, 6). The saccule, utricule, and ductus cochlearis were dissected from 2- to 7-day-old newborn chicks, and hair cells were dissociated enzymatically. After decapitation and sagital sectioning of the head, the bony labyrinth was exposed by removing most of the brain. The membraneous labyrinth was obtained, and the portions facing to the otoconia were cut into small pieces with microscissors and were incubated at 37°C for 40 to 50 min in Ca/Mg-free balanced salt solution (137 mM NaCl/5.4 mM KCl/0.2 mM Na_2HPO_4/0.4 mM KH_2PO_4/4.2 mM $NaHCO_3$/10 mM glucose) with 7–8 microunits/ml of papain (Sigma, P-3125), buffered to pH 7.4 with 20 mM Hepes/NaOH. They then were washed several times with Dulbecco-modified Eagle's essential medium, triturated, and plated on the lectin-coated glass floor of the recording chamber. Serum and antibiotics were not used in any of the steps described above. More than 50 hair cells with a good-looking hair bundle could be obtained from one animal. Most of the cells used in the experiments did not have a kinocilium. Hair cells were bathed physiologically in an asymmetrical ionic environment; their apical surfaces were facing K-rich/low-Na endolymph solution, while their basal surfaces were surrounded by supporting cells and were facing Na-rich/low-K perilymph solution (7). Normal saline used here has an ionic composition similar to the perilymph solution: 155 mM NaCl/5 mM KCl/2.5 mM $CaCl_2$/1 mM $MgCl_2$ buffered to pH 7.4 by 10 mM Hepes/NaOH.

Patch electrodes of 5–10 MΩ resistance were filled with isotonic CsCl solution, and a whole-cell recording variation of the patch-clamp technique was applied to the isolated hair cell, following the procedure described by Hagiwara and Ohmori (8). After the whole-cell recording condition was established, movements of the patch electrode for distances of 2–3 μm caused a distortion of the cell body but did not cause any change in the holding current. Thus, this technique seemed ideal for the study of the mechanoelectrical transduction of hair cells because it is necessary to apply mechanical stimuli to the hair bundle.

Mechanical stimulation was applied to the hair bundle by a glass rod with a fine tip, which was attached to a piezoelectrical device (9). A triangular electrical driving signal of 3–10 Hz was applied to the piezoelectrical stimulater, and the tip of the glass rod was moved in the horizontal plane with an amplitude of 0.5–2.5 μm. In order to protect the hair cells from damage resulting from a mechanical drift of the stimulating glass rod, the glass rod was placed 1–2 μm away from the hair bundle, and its position was frequently adjusted. The tips of the hairs were moved 1–2 μm at one end of the periodical motion of the glass rod. Direct stimulation of the cell body did not cause phasic changes of the holding current that followed the driving signal, but it occasionally killed the cell. Phasic changes of the whole-cell current have never been observed unless hairs were directly stimulated. When the cell body made a pivotal motion about the tip of the patch electrode, even though hairs were apparently moved, transduction currents were not observed. Therefore, the effective mechanical stimulus for generation of the transduction current was a movement of the hair bundle relative to the cell body. The orientation of the stimulus applied to the hair bundle was not always ideal because the dissociated hair cells settled on the glass floor with random orientation and the access of the stimulating glass rod was limited. The movement of the hair bundle and individual stereocilium was confirmed during experiments by observation with Nomarsky differential interference optics (overall magnification, ×600). The recording chamber was constantly perfused with heated saline; the bath temperature was maintained between 29 and 31°C.

RESULTS

Mechanoelectrical transduction currents were recorded from 27 hair cells. When direct mechanical stimulation was applied to the hair bundle, transient inward currents synchronized to the driving signal were observed (Fig. 1*A*). The peak of the inward current coincided with the peak of the electrical driving signal applied to the piezoelectrical device. Thus, the probe must have followed the driving signal, and the hair bundle was stimulated in phase with the movement

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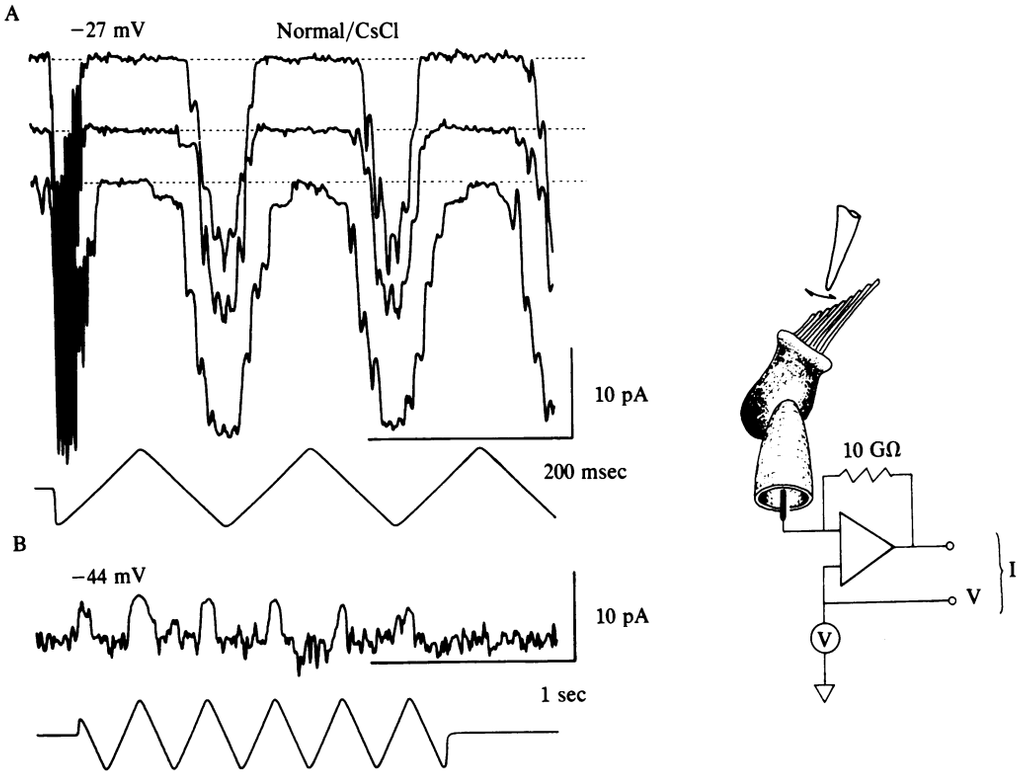

FIG. 1. Mechanoelectrical transduction currents in a single chick vestibular hair cell. (*A*) When hairs were directly stimulated by a glass rod, stepwise changes of inward currents were observed at −27 mV. Three traces are shown here, displaced vertically. Dashed lines indicate the levels of the holding current. Hair cells were bathed in normal saline, and the patch electrode was filled with 160 mM CsCl/5 mM EGTA solution buffered to pH 7.4 by 10 mM Hepes/NaOH. The electrical driving signal applied to the piezoelectrical stimulator is indicated in *A* and *B* by triangular waves. Stimulating frequency was 6 Hz. Because the transduction currents were generated in synchrony with the driving triangular wave, the probe must have followed closely the 6-Hz driving signal. Transduction currents were converted to voltage signals by an I–V converter with a 10-GΩ feedback resistor (*Inset*) and were low-pass-filtered at 800 Hz. Current and potential outputs of the patch-clamp amplifier and the driving signal were sampled at 1-msec intervals. (*B*) Membrane was hyperpolarized to −44 mV, and the stimulating probe was moved to the opposite side of the hair bundle from that of Fig. 1*A*. Note a decrease of the steady-state inward current synchronized to the driving signal, which occurs at the opposite phase of the probe motion to the case in *A*. The stimulating frequency was 3 Hz, and the signal was sampled at 5-msec intervals after filtering at 160 Hz. Records *A* and *B* were taken from a single cell at 29°C. (*Inset*) Arrangement of a stimulating probe and a patch electrode to a hair cell.

of the glass rod. Close inspection of the current traces reveals stepwise change of the current, suggesting that the whole-cell transduction current was composed of discrete levels of current amplitude. At each step of the current, an "overshooting" was observed. This may be due to a fluctuation in the number of activated transduction channels, distorted by the low pass filtering of the current. The initial burst of inward current in Fig. 1*A* was most likely due to a rapid movement of the glass rod from its resting position because the driving signal changed abruptly at the start of stimulation. When the position of the stimulating glass rod was moved to the opposite side of the hair bundle from that in Fig. 1*A*, an outward transient current was generated (Fig. 1*B*). The current traces indicate that the outward transient was generated by movement of the hair bundle in the opposite direction to that which produced inward current in the case of Fig. 1*A*.

This change of the current in the outward direction was a decrease of the steady-state inward current and not a generation of an outward current because (*i*) the reversal potential of the transduction current was +8.5 mV under these experimental conditions (normal saline as the bathing medium; isotonic CsCl as the intracellular medium) and (*ii*) a decrease of current noise was observed during the phase of the outward transition, which might indicate a reduced number of open channels. The transduction current disappeared reversibly by an application of 100 μM streptomycin sulfate (Sigma, S-6501) to the bathing solution. These results confirmed previous observations of the permeability of the hair-cell membrane to monovalent cation and the effect of streptomycin on the mechanoelectrical transduction channel that was made on microphonic potentials, on extracellular transduction current during direct mechanical stimulation to the hair, and on the transduction current measured during two-microelectrode voltage clamp of the hair cell (10–12).

From current traces in Fig. 1*A*, a probability density histogram of the event amplitude was measured (Fig. 2), excluding the initial burst-like current response. The tallest peak at the zero-current level indicates background fluctuations of the whole-cell current; SD = 0.4 pA. The second peak (indicated by a filled triangle) was at −1.75 pA and corresponds to the step changes of the transduction current observed at the base of the response. The histogram does not reveal clear peaks at integer multiples of −1.75 pA (indicated by open triangles), although some of the peaks coincide with the amplitude indicated by open triangles. This may be due to a limited signal-to-noise ratio resulting from the whole-cell recording condition and to the limited overall frequency response of the recording system; the current was low-pass-filtered at 800 Hz.

Step-like changes of the transduction current can be observed more clearly in Fig. 3. The phasic response of the transduction current to a triangular driving signal followed discrete steps of approximately 7 pA (indicated in the figure by broken lines). However, 7 pA may not correspond to the elementary step because smaller current steps of approxi-

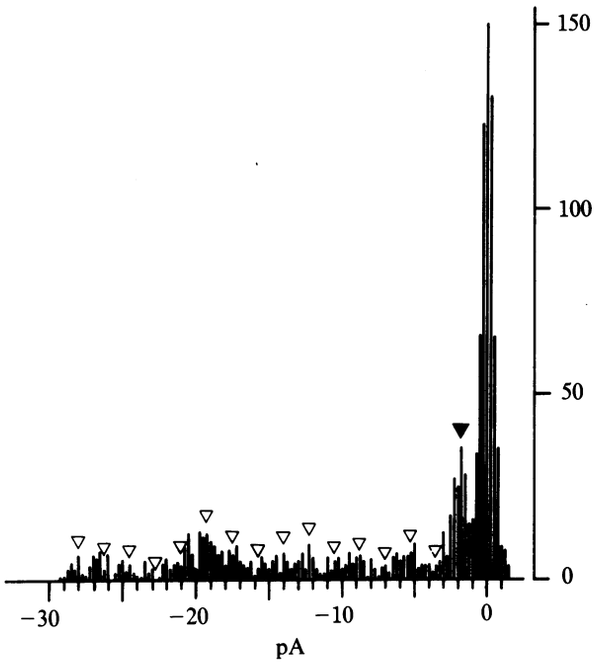

FIG. 2. Probability-density histogram of current amplitude. The displacement of current signals from the base line was measured at each time point from traces in Fig. 1A. In the measurements, the initial burst-like current transient was excluded. The ordinate is the number of events, and the abscissa is the event amplitude. The experiment was performed in normal saline, and the reversal potential of the transduction current was +8.5 mV; thus, the conductance that carries −1.75 pA at −27 mV is 49 pS. ▼, Peak at −1.75 pA; ▽, current levels corresponding to the integer multiples of −1.75 pA. Bin width is 0.25 pA.

mately half this amplitude are clearly seen at the top of each transduction current response and during the falling phase of the third response. Therefore, the levels indicated by broken lines in Fig. 3 may correspond to the coincidental opening of at least two elementary transduction channels. The conductance calculated from the current amplitude indicated by the broken line was 110 pS, which is approximately twice the conductance calculated from the peak detected in the histogram (49 pS, Fig. 2). In other experiments, apparent elementary conductances of 47–53 pS were measured.

DISCUSSION

Hudspeth has argued that a single stereocilium may have a single transduction channel (2), and it is known that a single hair cell has 30–150 stereocilia on its apical surface (13–17). By using a value of 50 pS for the single transduction conductance, this would predict a maximum conductance increase of 1.5–7.5 nS during a transduction response. The maximum response observed in the present experiments was 0.9 nS. This smaller value may result (*i*) from the loss of the transducer activity in a significant number of stereocilia during the drastic procedure of cell dissociation or (*ii*) from a smaller value of the single transduction conductance itself than the value we found. If we assume the second possibility, the apparent elementary step observed here could be a coincidence of a number of smaller single transduction-current changes. If such a coincidence ever happened, it might be related to the step-like changes of the height of stereocilia, and the apparent elementary current level that we observed might have been generated by a concerted activity of a number of stereocilia of the same height.

Stereocilia are known to be interconnected by a filamentous attachment (18–20). Such an attachment could cause a movement of stereocilia *en masse* in response to a mechanical stimulation (21). However, it seems likely that the position of each stereocilium can fluctuate somewhat within its filamentous attachment and would result in a fluctuation of the number of transduction channels activated. This would partly explain the "overshooting" response observed in Fig. 1A. The overshooting response also could be generated by a slight deviation of the probe motion from the axis of bilateral

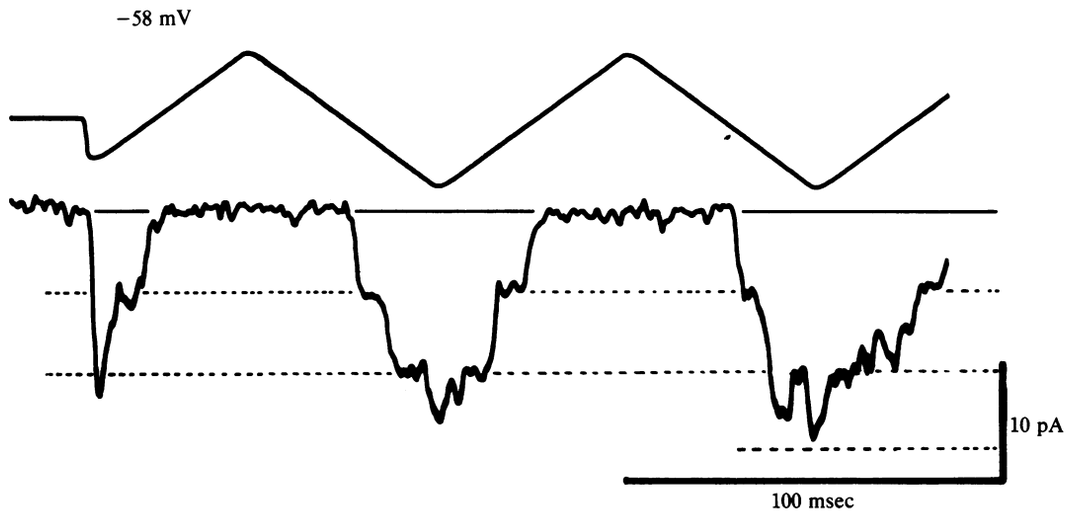

FIG. 3. Transduction current showing discrete levels of current. Discrete current levels were generated at −58 mV in response to a mechanical stimulation of the hair. Despite an abrupt change of the driving signal at the beginning of stimulation, burst-like transduction current was not generated in this case. The driving signal indicates that the probe stimulated the hair bundle only at the very end of its excursion, suggesting that the initial position of the stimulating probe was further from the hair bundle than in the case in Fig. 1A. Because the number of transduction channels mobilized seems much smaller here than in the case of Fig. 1A, discrete current steps were more obvious here (dashed lines). The experiment was performed in 160 mM CsCl/2.5 mM $CaCl_2$/1 mM $MgCl_2$ buffered to pH 7.4 by 10 mM Hepes/tetramethylammonium·OH; 160 mM $CsCl_2$/5 mM EGTA was used in the electrode. The reversal potential of the transduction current was +4.5 mV. Note smaller step changes at the peak of transduction current responses and during the falling phase of the third response. The amplitude of these smaller signals was approximately half the amplitude indicated by the broken lines. It is likely that the step levels indicated here were generated by a coincidental activity of at least two elementary transduction channels. Signals were low pass filtered at 800 Hz and were sampled at 500 μsec intervals. The experiment was performed at 31°C.

symmetry in the hair bundle resulting from the stepwise change of the height of stereocilium. Because the hair bundle is pushed by a glass rod in the present experiment, if the direction of the probe motion were slightly deviated from the axis of the hair bundle's bilateral symmetry, individual stereocilium in a rank would make a ratchet-like interaction with the probe tip and would generate spiky appearance in the macroscopic transduction current. The presence of the filamentous attachment between individual stereocilium is likely to cause a concerted motion of stereocilia in a rank and would have generated the step level of 7 pA, and the ratchet-like interaction between the probe and the individual stereocilium might have generated the intermediate current amplitude observed in Fig. 3. As a conclusion, the data presented in this paper strongly suggest that the mechanoelectrical transducer has discrete conductances with an elementary conductance of at most 50 pS.

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1. Flock, Å. (1971) in *Handbook of Sensory Physiology*, ed. Lowenstein, W. R. (Springer, Berlin), Vol. 1, pp. 396–441.
2. Hudspeth, A. J. (1983) *Annu. Rev. Neurosci.* **6,** 187–215.
3. Lewis, R. S. & Hudspeth, A. J. (1983) *Nature (London)* **304,** 538–541.
4. Ohmori, H. (1984) *J. Physiol.*, in press.
5. Lam, D. M. K. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1987–1991.
6. Bader, C. R., Bertrand, D. & Schwartz, E. A. (1982) *J. Physiol.* **331,** 253–284.
7. Peterson, S. K., Frishkopf, L. S., Lechéne, C., Oman, C. M. & Weiss, T. F. (1978) *J. Comp. Physiol.* **126,** 1–14.
8. Hagiwara, S. & Ohmori, H. (1982) *J. Physiol.* **331,** 231–252.
9. Corey, D. P. & Hudspeth, A. J. (1980) *J. Neurosci. Methods* **3,** 183–202.
10. Mastuura, S., Ikeda, K. & Furukawa, T. (1971) *Jpn. J. Physiol.* **21,** 579–590.
11. Corey, D. P. & Hudspeth, A. J. (1979) *Nature (London)* **281,** 675–677.
12. Hudspeth, A. J. (1982) *J. Neurosci.* **2,** 1–10.
13. Iurato, S. (1967) *Submicroscopic Structure of the Inner Ear* (Pergamon, Oxford).
14. Miller, M. R. (1978) *J. Morphol.* **156,** 381–418.
15. Kimura, R. S. (1966) *Acta Otolaryngol.* **61,** 55–72.
16. Takasaka, T. & Smith, C. A. (1971) *J. Ultrastruct. Res.* **35,** 20–65.
17. von Düring, M., Karduck, A. & Richter, H. G. (1974) *Z. Anat. Entwicklungsgesch.* **145,** 41–65.
18. Bagger-Sjöback, D. & Wersäll, J. (1973) *J. Neurocytol.* **2,** 329–350.
19. Bagger-Sjöback, D. (1974) *Brain Behav. Evol.* **10,** 88–94.
20. Hama, K. & Saito, K. (1981) *Adv. Neurol. Sci.* **25,** 756–776.
21. Flock, Å. (1977) in *Psychophysics and Physiology of Hearing*, eds. Evans, E. F. & Wilson, J. P. (Academic, New York), pp. 15–23.